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(54) Title: COMPOSITION AND METHOD FOR ADMINISTRATION OF BIO-AFFECTING CATALYSTS

(57) Abstract

Compositions and methods for administration of a bio-affecting catalyst, such as an enzyme, are provided. The compositions comprise at least one enzyme or other bio-affecting catalyst, retained within a matrix. The matrix provides a barrier between the entrapped enzyme and external degradative enzymes or cellular defense systems of a patient's body, and also prevents release of the enzyme. The matrix comprises pores or channels to enable entry and exit of substrates and products. Preferably, the matrix is a high viscosity cubic phase of one or more suitable amphiphilic compounds, such as glyceryl monooleate. Preferred methods of the invention comprise formulating the composition as an injectable low viscosity phase, which undergoes phase transition upon exposure to physiological conditions.

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COMPOSITION AND METHOD FOR ADMINISTRATION OF BIO-AFFECTING CATALYSTS

FIELD OF THE INVENTION

The present invention relates to treatment of diseases and other pathological conditions associated with enzyme defects or imbalances. In particular, the invention provides compositions and methods for supplying enzymes or other bio-affecting catalysts, for long-term enzyme replacement/augmentation therapy.

BACKGROUND OF THE INVENTION

To date, more than one hundred and fifty metabolic diseases have been ascribed to specific enzyme defects. These defects often result in physically and mentally crippling diseases. In many of the defects a specific enzyme is completely missing, while in others the enzyme is replaced with a relatively inactive isoenzyme. A small portion of the enzyme deficiency diseases can be treated by controlled diets (e.g., phenylketonuria, a defect in aromatic amino acid metabolism, can be treated by abstaining from foods containing phenylalanine). A potentially more generally applicable treatment strategy, however, is enzyme replacement/augmentation therapy wherein the missing enzyme is administered to a patient in order to alleviate or remove the symptoms of the disease.

In conventional enzyme replacement therapy, an exogenous enzyme is administered to the patient either intravenously or intramuscularly. One problem with the conventional therapy arises because enzymes are proteins and are therefore susceptible to rapid proteolytic degradation as well as elimination from the patient's body. Additionally, the exogenous enzyme may elicit undesirable immunological responses upon being introduced into the body of the patient. As a result, the patient's own defenses may actually attack and destroy the enzyme,

as well as eliciting detrimental effects to the patient associated with over-stimulation of the patient's immune system and other defense responses. A further disadvantage of conventional enzyme treatment arises due to the frequent requirement for the missing enzyme in localized regions of the body. Parenteral administration of the exogenous enzyme can fail to provide adequate therapeutic concentrations of the enzyme in the necessary regions of the body for long periods of time.

Thus, with conventional enzyme replacement therapy, the exogenous enzyme has a very short half-life in the body and may never reach its target location in appreciatable quantities. As a result, administration of large quantities of the enzyme is likely to be required. This requirement is inconvenient for the patient and is also impractical, since purified enzyme may not be available, or may be prohibitively expensive, in large enough quantities for long-term treatment.

Various methods and compositions have been proposed for improving delivery of therapeutic agents to their sites of action and for effecting long-term sustained release of therapeutic agents. A number of semi-solid ointments or gels have been shown to be effective for increasing drug retention times, especially in the case of ophthalmic drug delivery. Other compositions have been developed for administration via injection, which comprise polymeric delivery vehicles that are of low viscosity under preparatory conditions, but form a semi-solid gel when subjected to physiological conditions.

Lipophilic and amphiphilic compositions have also been utilized in sustained-released drug delivery systems. For example, U.S. Patent No. 5,230,895 to Czarnecki et al. describes a method and composition for treating periodontal disease with a therapeutic agent released in a sustained manner from a glyceride composition that forms a semi-solid gel when placed in

the periodontal pocket. U.S. Patent No. 5,151,272 to Engstrom et al. discloses a controlled-released composition for biologically active materials which comprises a gel-like liquid crystalline phase (e.g., lamellar, hexagonal, cubic and micellar). The biologically active substance is released into the body as a result of erosion of the liquid crystal phase or diffusion of the substance into the surrounding medium.

The above-described gels and liquid crystal matrices are all designed to deliver biologically active agents by releasing the agent into the body fluid or tissue into which the composition has been injected, implanted or otherwise administered. However, for enzyme replacement therapy, as discussed above, release into the body is detrimental for two reasons: first, it exposes the enzyme to proteolytic and immunological attack; second, it allows the enzyme to diffuse from the precise location in the body where it may be required. What is more, because enzymes are catalysts, their release into the body from a gel or liquid crystal matrix is unnecessary. It is necessary only that the enzyme be accessible to its appropriate substrates in the body, and that it be capable of exerting its required enzymatic function and releasing the enzymatic products back to the body where they are required.

Thus, it would be a significant advance in the art of enzyme replacement therapy to provide compositions and methods for supplying an enzyme (or other biologically relevant catalyst) to a patient in a form in which the enzyme is protected from proteolytic degradation and immunological attack, yet is accessible to entry and exit of substrates and products and is capable of performing the requisite enzymatic activity required by the patient. It is also desirable for such compositions and methods to provide therapeutic concentrations of a required enzyme in a localized region of the body. Preferably, the compositions should be

administrable by syringe and thereafter capable of forming a protective matrix surrounding the enzyme. Finally, the compositions should be slowly erodible, so as to eliminate the need for removal after the effective life of the enzyme is over.

SUMMARY OF THE INVENTION

The present invention provides compositions and methods for administration of bio-affecting catalysts. As used herein, the term "catalyst" retains its common definition as a substance that influences the speed of a chemical reaction without itself undergoing a permanent change. For purposes of the present invention, chemical reactions that occur in, or affect, biological systems are referred to as "bio-affecting" reactions, and "bio-affecting catalysts" are those that catalyze "bio-affecting" reactions. Bio-affecting catalysts may themselves be biological molecules, such as enzymes, but they need not be.

According to one aspect of the present invention, a composition is provided that comprises at least one bio-affecting catalyst retained within a matrix. The matrix functions to substantially prevent release of the bio-affecting catalyst therefrom for a pre-determined time period, preferably calculated relative to the effective lifespan of the catalyst. Catalysts retained within the matrix are in fluid communication with the external medium in which the composition is disposed, thereby enabling accessibility of the catalyst to substrates in the external medium, and release into the external medium of products formed by the catalyst. Preferably, the catalyst erodes substantially after expiration of the pre-determined time period.

The matrix is a high viscosity gel or liquid crystalline phase that not only functions to retain the bio-affecting catalyst, but also functions to protect

the bio-affecting catalyst from external proteolytic or other degradative enzymes and/or cellular defense mechanisms present in the physiological environment. Fluid communication through the matrix occurs as a result of the composition and structure of the matrix. High viscosity gel matrices comprise amorphous networks of polymeric molecules, the spaces therebetween providing channels for the fluid communication. High viscosity liquid crystalline matrices comprise semi-regular or regular arrays of amphiphilic molecules, separated by pores or channels for the fluid communication. Liquid crystalline phase high viscosity matrices are preferred in the present invention, with cubic phases being particularly preferred.

The above-described composition preferably is prepared as a low viscosity phase mixture comprising the bio-affecting catalyst and at least one matrix-forming compound, and can be induced to undergo a phase transition to a high viscosity phase to produce the matrix with the catalyst retained therein. The phase transition is inducible by exposing the low viscosity phase to changes in one or more conditions, including temperature, pH or solvent composition. In a preferred embodiment, the phase transition is inducible by introduction into the body of a patient.

According to another aspect of the present invention, a composition is provided that comprises at least one bio-affecting catalyst retained within a high viscosity cubic phase matrix. The composition is prepared as a low viscosity phase, comprising by weight between about 60% and about 99.99% of glyceryl monooelate, up to about 40% (more precisely, 39.99%) of an aqueous phase, and between about 0.01% and about 40% of one or more bio-affecting substances.

According to another aspect of the present invention, a method is provided for supplying a bio-affecting catalyst to a patient. The method comprises

administering to a patient a composition comprising at least one bio-affecting catalyst retained within a matrix, which (1) substantially prevents release of the catalyst therefrom for a pre-determined time period, and (2) enables fluid communication between the catalyst and an external medium in which the composition is disposed, resulting in accessibility of the catalyst to substrates in the external medium and release of products into the medium.

The above method preferably comprises formulating the composition as a low viscosity phase mixture, preferably injectable, which comprises the bio-affecting catalyst and one or more matrix-forming compounds. The mixture is then induced to undergo a phase transition to a high viscosity phase to produce the matrix with the catalyst retained therein. In a particularly preferred embodiment, the low viscosity phase mixture is injected into the patient at a selected location (e.g., intramuscularly, subcutaneously or into a desired tissue or organ), and the phase transition is induced by exposure of the mixture to the physiological conditions present at the injection site. In an alternative embodiment, the composition is administered by removing a component (e.g., body fluid or tissue) from a patient's body, exposing the component to the composition, then returning the component to the patient's body.

BRIEF DESCRIPTION OF THE DRAWINGS

The foregoing summary, as well as the following detailed description of the preferred embodiments of the present invention, will be better understood when read in conjunction with the accompanying drawings, in which:

Fig. 1 is a plot of the fraction of FITC-labelled horseradish peroxidase, FITC-labelled insulin, or methylene blue that has escaped from a cubic phase of glyceryl monooleate as a function of time;

Fig. 2 is a plot of the percent of horseradish peroxidase (HRP) activity as a function of time in the presence of lipase and/or trypsin; and

Fig. 3 is a plot of the percent of catalase activity as a function of time.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

In its most basic form, the composition of the present invention comprises a matrix in which is disposed a bio-affecting catalyst, such as an enzyme. The composition is initially formed as a mixture of the matrix-forming compound(s) and bio-affecting catalyst(s) wherein the mixture exists as a low viscosity phase. However, the mixture is capable of undergoing a phase transition to a high viscosity phase, such as a liquid crystal or gel, with the bio-affecting catalyst entrapped within. The high viscosity phase matrix comprises pores or channels that allow for the ingress and egress of substrates and products to and from the catalyst disposed therein. However, the size of the pores or channels is sufficiently small to impede or prevent diffusion of the catalyst from the matrix, and to protect the catalyst from proteolytic enzymes and cells of the immunological response system.

The present invention can be practiced with a variety of bio-affecting catalysts. These catalysts may be naturally-occurring enzymes or enzyme derivatives, or they may be any other catalyst (e.g., catalytic peptides or peptide derivatives) useful for producing biological products normally supplied by a naturally-occurring enzyme in a patient's body. Enzyme classes that may be utilized in the present invention include, but are not limited to, oxidoreductases, transferases, hydrolases, lyases, isomerases, and ligases (or synthetases). Examples of enzymes suitable for use in the present invention, include but are not limited to, the enzymes set forth in Table "A" below.

Table A

Enzyme	Intended Use	Comments
L-asparaginase	Acute lymphoblastic leukemia	Substrate: Asparagine Mechanism: deprivation of substrate needed by tumor
L-glutaminase	Neoplasms	Substrate: Glutamine Mechanism: deprivation of substrate needed by tumor
Urease	Uremia	Substrate: Urea
α-galactosidase	Fabry's disease or Ceramide-trihexosidosis	Substrates: gal-gal- glucosyl ceramide, and digalactosyl ceremide
Adenosine deaminase	Severe combined immuno-deficiency (SCID)	
Hexosaminidases A and B	Sandoff's disease	Substrates: GM2 ganglioside, asilo-GM2 ganglioside, and globoside
β-N-acetyl hexosaminidases A	Tay-Sachs disease	Substrates: GM2 ganglioside and asilo-Gm2 ganglioside
Glucosyl cerebramidase and β-glucocerebrosidase	Gaucher's disease	Substrates: Glucosyl-ceramide, glucosyl-sphingosive, and sialyl lactosylceramide

In addition, one or more bio-affecting catalyst can be simultaneously entrapped within the matrix. If more than one catalyst is entrapped, they can act independently or as co-catalysts in a complex system. The matrix may further comprise coenzymes and other cofactors required to accomplish complex bio-catalytic functions.

As described above, the matrix possesses pores or channels that allow for the movement of substrates and products to and from the enzyme and the external environment. The pores or channels are sufficiently narrow to prevent or impede the bio-affecting catalyst from diffusing out of the matrix. Retention may also be facilitated by partitioning of the catalyst with the lipid phase of the matrix (preferred matrices of the invention comprise a lipid phase). Under certain circumstances (e.g., for catalysts with low molecular weights or for small co-factors), it may be desirable to further restrict the rate at which the bio-affecting catalyst diffuses from the matrix. This may be accomplished by attaching the catalyst to (or combining it with) an anchor molecule that increases the molecular weight of the catalyst without materially affecting its function, or that increases the affinity of the catalyst for the matrix (e.g., by facilitating partitioning with a lipid phase of the matrix). Examples of anchor molecules include, but are not limited to: l-palmitic acid and other fatty acids, peptides, proteins, polyethylene glycol and other polymers, or a combination of such molecules.

The bio-affecting catalyst is disposed within a matrix comprising pores or channels of a size that enable entry/exit of substrates/products, while concomitantly preventing exit of the bio-affecting catalyst during its functional lifetime and substantially preventing entry of proteolytic or other enzymes or immunological defense cells of the body. The pore or channel size may vary, but is preferably within the range of 0.1 to 100 nm, more preferably between 1 and 20 nm, and most preferably on the average of 5 nm. As discussed in greater detail below, the matrix preferably comprises a relatively uniform channel structure, that may be adjusted by varying the composition and relative concentrations of components used to prepare the matrix. Optimum size

ranges are formulated depending on the size of the bio-affecting catalyst, as well as sizes of substrates and product molecules.

Preferably, the matrix should be made from one or more matrix-forming compounds that can be mixed with the bio-affecting catalyst as a low viscosity phase, to enable even distribution of the bio-affecting catalyst in the matrix to be formed. The mixture then should be capable of undergoing a phase transition to a high viscosity phase, thereby forming the matrix in which the bio-affecting catalyst is disposed. The transition from the low viscosity phase to the high viscosity phase is effectuated, for example, by a change in pH, temperature, and/or solvent composition (e.g., by contacting an alcoholic or lipid mixture with water, ionic amphiphiles or salts). The phase transition can be induced *ex vivo*, prior to administration into the body of a patient, and thereafter be introduced into the body by implantation. However, it is preferred to prepare the composition as a low viscosity phase injectable mixture, which undergoes a phase transition to the high viscosity phase upon being exposed to one or more physiological conditions (e.g., solvent change, pH change, temperature change, as described above). In this preferred embodiment, the composition is injected as a low-viscosity phase mixture through a syringe, so that it can be easily placed virtually anywhere within the patient's body, and thereafter undergoes a phase transition to form the high viscosity matrix in which the bio-affecting catalyst is entrapped.

A variety of compounds are available that can be induced to exhibit a phase transition from a low to high viscosity phase. These include reversible and irreversible gel-forming compounds, such as those described in co-pending U.S. Patent Application Serial Nos. 08/261,731 and 08/287,694 (the disclosures of which are incorporated herein by reference in their entireties)

and references cited therein (e.g., U.S. Patent Nos. 4,188,373, 4,474,751, 4,474,752, 5,292,517 and 5,252,318). The high viscosity phase formed by these reversibly-gelling compositions are gels comprising amorphorous polymer networks in a substantially aqueous environment. Preferred gels comprise low molecular weight polymers (e.g., mw < 50,000), which will slowly dissolve in the body, or polymers that are otherwise degradable in the body, to eliminate the need for surgical removal.

Although gels can be used, it has been discovered in accordance with the present invention that matrices having well structured pores or channels provide better accessibility of the bio-affecting catalysts to substrates (and better release of products) than do gel matrices with amorphous polymer networks, or other matrices having poorly formed channel structures. Accordingly, preferred for use in the present invention are compounds capable of undergoing a phase transition to a liquid crystal structure, such as a cubic, hexagonal or reverse hexagonal phase. Of these, cubic phases are most preferred because of their uniform pore/channel structure.

Compounds that may be induced to undergo phase transitions to liquid crystal phases are known in the art (see, e.g., Fontell, Colloid and Polymer Science 266: 264-285, 1990). Liquid crystalline phases are common in amphiphilic lipid systems, including compositions comprising simple surfactants, straight- or branched-chain lipids and/or complex biological lipid mixtures. Some amphiphilic lipid systems are capable of undergoing a phase transition, e.g., upon temperature increase from a low viscosity lamellar phase (i.e., a phase formed by lipid-water systems in which lipid bilayers alternate with water layers) to a high viscosity liquid crystalline phase. The liquid crystalline phase may be a cubic phase (i.e., an isotropic phase with a three-dimensional

structure in which the lipid bilayer forms a continuous structure that separates two identical networks of water channels), a hexagonal phase (lipids forming tubular micelles with hydrophobic tails facing internally) or reverse hexagonal phase (tubular micelles with hydrophilic heads facing internally).

Compounds capable of undergoing a phase transition to form liquid crystalline phases have been explored for use in sustained-release drug delivery systems (see, e.g., U.S. Patent 5,151,272 and 5,230,895; see also Engström et al., Int. J. Pharmaceutics 86: 137-145, 1992; Norling et al., J. Clin. Peridontol. 19: 687-692, 1992). However, the compositions disclosed therein were utilized not for their pore/channel structure but instead for their ability to be combined with either polar or nonpolar drugs. Furthermore, the disclosed compositions were formulated for the purpose of releasing drugs or other bio-active compounds into the body, not for retention of a bio-affecting catalyst, for purposes of protecting the catalyst while enabling entry and exit of substrates and products, as disclosed herein in accordance with the present invention.

Thus, compositions of the invention are preferably formulated from the following general classes of compounds at the indicated preferred concentration ranges:

1. one or more amphiphilic matrix-forming carrier compounds: approx. 1% - 99.99% (a 10% minimum is preferred);

2. aqueous phase (water, salt solution, buffer, etc.): approx. 0% - 90.0% (A formulation in which the mixture contains no initial aqueous phase may be prepared. Such a formulation will form the liquid crystal phase upon administration into an aqueous environment, such as the body, the transition induced by absorption of water and/or temperature increase from room temperature to body temperature.);

3. bio-affecting catalysts, co-catalysts and/or co-factors: in appropriate effective concentrations, preferably about 0.01% - 50% (but could be less or greater depending on the specific catalysts and other substances used);

4. other ingredients: approx. 0.01% - 25%.

Amphiphilic molecules that may be utilized in preparing compositions of the invention include, but are not limited to: lipids, surfactants and soaps capable of forming high viscosity liquid crystalline phases. These components may be used alone or in combination.

Nonlimiting examples of lipids that may be utilized in the compositions of the invention include glycerides, phospholipids, fatty acids and sphingolipids. Glycerides include, but are not limited to, monoglycerides, diglycerides and triglycerides. In a preferred embodiment the monoglyceride, glyceryl mono(cis-9)oleate (also referred to as glyceryl monooleate), is used, as described in greater detail below.

Nonlimiting examples of soaps that may be incorporated into compositions of the invention include anionic and cationic soaps. Examples of surfactants include, but are not limited to, nonionic and zwitterionic surfactants.

Nonlimiting examples of suitable aqueous media to be used in formulating the composition of the invention include water, various biologically acceptable buffers (e.g., phosphate buffers, Tris buffers and other similar physiological buffers) and isotonic solutions containing salts. In some embodiments, the aqueous medium may contain a biologically acceptable alcohol, such as ethanol, for purposes of improving the solubility of polymers in the low viscosity phase (see, e.g., U.S. Patent Application No. 08/261,731, the disclosure of which is incorporated herein by reference).

Examples of bio-affecting catalysts have been discussed in detail hereinabove. Compositions of the invention may contain one or more such bio-affecting catalysts, along with co-catalysts and co-factors, as required.

Other ingredients may also be included in compositions of the invention for various purposes, such as to modify the structure of the high viscosity phase and/or to accelerate or decelerate degradation of the matrix. These include, but are not limited to, glycerol and other similar solvents, lipids (e.g., glycerides, phospholipids, sphingolipids, cholesterol, fatty acids, etc.), oils, proteins and other biologically compatible molecules. As used herein with reference to the several components of the compositions of the invention, the expression "biologically acceptable" or "biologically compatible" refers to substances which do not adversely affect the activity or efficacy of the bio-affecting catalyst and other bio-active substances included in the compositions, and which are not in themselves toxic to the recipient. In accordance with this definition, other components that may be included in the compositions of the invention include, e.g., preservatives, protein stabilizers, enzyme inhibitors (e.g., lipase inhibitors, esterase inhibitors, protease inhibitors), as well as ingredients for stabilizing enzymes, reducing escape of enzymes from the matrix, agents that modify the structure of the matrix (e.g., by modifying the pore or channel size or by modifying the rate of degradation of the matrix).

In a preferred embodiment, the matrix in its high viscosity phase should be slowly erodible in a physiological environment. For purposes of this invention, the term "erodible" means that (1) the matrix is susceptible to degradation by enzymes and/or other agents naturally occurring in a physiological environment such as a patient's body, or (2) the matrix slowly

dissolves, or (3) the matrix erodes by a combination of dissolution, degradation and/or other means. An erodible matrix eliminates the need for removal of the composition upon completion of the treatment. If an erodible matrix is utilized, the rate of erosion should be adjusted so that the matrix erodes substantially after the effective life span of the bio-affecting catalyst is completed. In a preferred embodiment, the rate of lipid degradation can be delayed by adding lipase inhibitors to the matrix-forming mixture in its low viscosity phase. The inclusion of lipase inhibitors functions to reduce to activity of lipases that may attack the lipid-containing matrix of the composition. As a result, the useful lifetime of the compositions are increased.

An exemplary composition of the invention comprises, as a matrix-forming compound, the polar lipid, glyceryl monooleate (hereinafter GMO), also known as monoolein. At 0-40% (preferably 10-20%) (by weight) water in GMO, the mixture forms a low viscosity, opaque lamellar phase in which bio-affecting catalysts and other ingredients may be solubilized. The viscosity of the lamellar phase is low enough that the GMO-catalyst mixture can be passed through a syringe. Upon injection into a patient's body, the mixture undergoes a phase transition to a high viscosity, clear cubic phase (approximately 10^5 centipoise, or greater). The phase transition is induced by the absorption of water and increase in temperature (from room temperature to body temperature). The cubic phase gradually erodes in the body, eliminating the need for surgical removal.

The GMO cubic phase comprises aqueous channels with average diameters of about 5 nm, which allow substrates and product to migrate to and from the bio-affecting catalyst (which, in this preferred embodiment, is a naturally-occurring enzyme or combination of enzymes). In addition, the cubic phase stabilizes the enzyme and protects the enzyme from proteases,

macrophages and other macromolecules and cells involved in the patient's immune or defense systems. The channels are sufficiently narrow so that enzymes are entrapped in the cubic phase, and are substantially prevented from exiting the cubic phase. As discussed above, under certain circumstances it may be desirable to further reduce the rate at which the enzyme (or other co-factors or bio-affecting catalysts) escape from the cubic phase. This may be accomplished by conjugating the enzyme to (or otherwise combining it with) an anchor molecule that increases the molecular weight or the lipid solubility of the enzyme without denaturing the enzyme. If the anchor molecule is hydrophobic, the interaction between the enzyme and the lipid bilayers of the matrix will be enhanced and will further decrease the rate of escape of the enzymes from the matrix. As described above, other kinds of molecules (proteins, polymers, etc.) may also be used to anchor the enzyme or to increase its affinity for the matrix.

The present invention also provides methods of using the compositions described above. In one method, the compositions are used for replacing or supplementing at least one enzymatic function in a patient. For purposes of the present invention the term "patient" refers to both humans and animals. The enzyme supplementation therapy may be accomplished by providing the deficient naturally occurring enzyme; alternatively, an enzyme derivative or synthetic substitute catalyst may be utilized to supply the enzymatic function deficient in the patient. According to the method, a mixture is prepared of the enzyme or other bio-affecting catalyst and the matrix-forming compound(s), along with other desired ingredients. The mixture is prepared under conditions whereby the mixture forms a low viscosity phase with the enzyme solubilized within the phase. The mixture is then subjected to conditions that induce a phase transition to a high viscosity phase, preferably an

ordered liquid crystalline phase. In one embodiment, the phase transition is induced *ex vivo*, and the resultant matrix-enzyme composition is administered to the patient by implantation into the desired location of the patient's body.

In a preferred embodiment, the mixture is administered to the patient by placing the mixture in a syringe and injecting it into the patient intramuscularly, subcutaneously or into a selected tissue. The phase transition is induced by the physiological conditions within the patient's body, including increased temperature and exposure to water. In this embodiment, the mixture also may be injected into a predetermined region of the body, so as to provide increased concentrations of the enzyme or other bio-affecting catalyst in that area.

The general method described above is applicable not only to enzyme replacement/augmentation therapy, but may also be used for treatment of substrate-dependent tumors. One example of a substrate-dependent tumor is human acute lymphoblastic leukemia. Lymphoblastic leukemia cells are deficient in L-asparagine synthetase and depend on exogenous supplies of L-asparagine formed in surrounding normal cells. Current therapy for lymphoblastic leukemia involves intravenous administration of the enzyme, L-asparaginase. L-asparaginase degrades L-asparagine, thereby depleting the body of L-asparagine. This causes the malignant cells to die of starvation due to lack of adequate supply of L-asparagine, which is an essential amino acid. Intravenous administration of L-asparaginase is not particularly effective because the enzyme is rapidly cleared from the bloodstream and also elicits undesirable immunological responses in the patient's body.

The compositions of the invention may provide a more effective treatment for substrate-dependent tumors. For example, injection of a composition comprising L-

asparaginase into a tissue or organ having high blood flow (e.g. spleen, liver, subcutaneous injection) will result in an increased half-life of the enzyme as well as sequestering it from recognition by the immune response system. Thus, removal of L-asparagine from the patient's body may be effected over a longer term, with administration of less L-asparaginase. Compositions of the invention should be particularly effective in the case of substrate-dependent solid tumors, where they can be injected near or in the tumor to deplete malignant cells of a required substrate.

The compositions of the invention may also be utilized for detoxification of various body fluids and tissues, both *in vivo* and *ex vivo*. In accordance with the general methods described above, compositions of the invention may be formulated in which the bio-affecting catalyst (e.g., comprising glutamine synthetase) functions to convert a toxic substance (e.g., ammonia) to a non-toxic substance (e.g., amino acids such as glutamine). Such compositions may also be used in extracorporeal shunting or filtering devices, such as dialysis devices or circulation devices in which a patient's blood is directed out of the body, through external tubing and back into the body. Compositions of the invention may be placed in such devices so that a selected body fluid comes in contact with the composition during its flow through the extracorporeal device. Compositions formulated to comprise bio-affecting catalyst having detoxification functions may be used to advantage to purge these body fluids of an unwanted or toxic substance during such procedures.

The following examples are provided to describe the invention in further detail. These examples are intended merely to illustrate the specific embodiments of the compositions and methods of the invention, and should in no way be construed as limiting the invention.

EXAMPLES

In order to demonstrate the efficacy of the compositions and methods of the present invention, mixtures of a matrix and various enzymes or complex enzyme systems were prepared. Myverol™ 18-99 glyceryl monooleate (GMO) (obtained from Eastman Chemical Company), a high quality distilled monoglyceride containing a minimum of 90 % assay as a monoester, was used as the matrix. The enzyme systems tested include horseradish peroxidase, catalase, and a combination of human cytochrome P450-2A6, cytochrome P450 reductase, and glucose-6-phosphate dehydrogenase.

Examples 1-7
Horseradish Peroxidase

Example 1: Phase Change

The activity of horseradish peroxidase (hereinafter, HRP) was tested using 2,2'-azino-bis-[3-ethylbenzthiazoline-6-sulfonic acid] (hereinafter, ABTS) as the substrate. In the presence of H_2O_2, HRP oxidizes ABTS to a radical cation which is deep bluish-green in color and has an absorption maximum at 410 nm. The concentration of product in solution is measured using spectrophotometric analysis. In addition, HRP concentrations in solution can be determined using fluorescein isothiocyanate-labelled HRP (hereinafter, HRP-FITC), which can be detected fluorimetrically at an excitation wavelength of 490 nm and an emission wavelength of 515 nm.

The samples of lamellar and cubic phase GMO containing HRP (or HRP-FITC) used in the examples were prepared as follows. A 10 mM solution of phosphate buffer (pH 7.4) containing HRP was mixed with GMO such that the final composition was, in weight percent, 85.00% GMO, 14.87% buffer, and 0.13% HRP. The mixture was stored at room temperature for 24 hours to form the

lamellar phase. The lamellar phase was then exposed to a 10 mM solution of phosphate buffer (pH 7.4) at 37°C, in order to simulate physiological conditions. Each gram of the lamellar phase absorbed about 0.3 grams of buffer and formed the cubic phase with a composition, in weight percent, of about 65.0% GMO, 34.9% buffer, and 0.1% HRP. Thus, the presence of the enzyme did not affect the formation of the lamellar phase or the transition to the cubic phase upon exposure to simulated physiological conditions.

Example 2: Enzyme Retention in the Cubic Phase

To determine if enzymes dissolved in the lamellar phase became entrapped in the cubic phase, 0.77 grams of the lamellar phase containing HRP-FITC was placed via syringe into each of three plastic containers (14 mm inner diameter and 7 mm deep). Each container was then placed in a separate beaker containing 30.0 mL of a 10 mM phosphate buffer solution (pH 7.4) at 37°C. Each beaker was placed on a magnetic stir-plate inside an incubator at 37°C. Exposure of the lamellar phase to the buffer solutions at 37°C caused a phase transition to the cubic phase. The solutions were stirred with magnetic stirring bars, with 1 mL of aliquots solution being withdrawn from each of the beakers at selected time intervals and replaced by 1 mL of buffer solution. The aliquots were subjected to fluorimetric analysis to determine the HRP-FITC concentrations. For comparison purposes, the retention of methylene blue (mw = 374 Da) and FITC-labelled insulin (mw = 6,000 Da) in a GMO cubic phase were determined in an analogous manner. The results are listed in Table 1 and shown in Fig. 1.

The data in Table 1 and Fig. 1 show that there was no detectable loss of enzyme from the lamellar phase as it transformed into the cubic phase. Further, at the end of 22 days about 55% of the HRP-FITC was still retained in the cubic phase. The results also indicate

that the extent and rate of escape of entrapped molecules appears to be molecular weight dependent (HRP (44,000 Da) < Insulin (6,000 Da) < Methylene blue (374 Da)). Thus, high molecular weight enzymes will tend to be better entrapped in the cubic phase without the use of anchor molecules.

Table 1

Time (days)	Fraction Escaped		
	HRP-FITC	Insulin-FITC	Methylene Blue
0	0.000	0.000	0.000
0.08	0.030	0.060	0.120
0.17	0.050	0.083	0.150
0.50	0.076	0.130	0.260
1	0.100	0.200	0.392
1.5	---	---	0.460
2	0.149	0.280	0.530
3	---	---	0.650
4	0.200	0.389	0.740
5	---	---	0.820
6	0.245	0.483	0.920
8	0.279	0.563	0.960
10	0.310	0.630	0.973
14	0.370	0.750	0.980
18	0.419	0.850	---
22	0.460	0.930	---

Example 3: Activity of Immobilized Enzyme in Cubic Phase

To determine whether the enzymes entrapped inside the cubic phase retained enzymatic activity, 1 gram of the cubic phase containing HRP was placed in a beaker and thoroughly rinsed with phosphate buffer solution to remove any surface associated HRP. A sufficient volume of a substrate solution containing 0.25 mM ABTS and 0.05 mM H_2O_2 was poured into the beaker to submerge the cubic phase. The color of the substrate solution began to change to a bluish-green, indicating the formation of product. Thus, the enzymes entrapped in the cubic phase retain their enzymatic activity.

The cubic phase was then removed from the beaker and thoroughly rinsed. The outer layers of the cubic phase were removed from all sides and a small portion of the core of the cubic phase weighing about 0.1 grams was placed in a beaker. The cubic phase was then treated with a substrate solution in the same manner described in the previous paragraph. The color of the substrate solution changed to a bluish-green, again indicating the formation of product. The activity demonstrated by the core removed from the interior of the cubic phase indicates that the enzyme is homogeneously distributed throughout the cubic phase and that the enzyme is not denatured within the cubic phase.

Example 4: Mechanism of Enzyme Action

To determine if the substrate can diffuse into the cubic phase and react with the enzyme immobilized inside the cubic phase, 1 gram of cubic phase containing HRP was placed in a glass vial (12 mm inner diameter), and the vial was centrifuged at 10,000 rpm so that the cubic phase formed a flat layer at the bottom of the vial. A 1 gram aliquot of GMO was poured on top of the cubic phase layer, followed by the addition of about 1 mL of a 10 mM phosphate buffer solution. The vial was left undisturbed for 24 hours. During that time, the opaque

GMO layer absorbed some of the buffer solution and transformed into the clear cubic phase on top of the cubic layer containing HRP. The remaining buffer solution was removed and 4 mL of a substrate solution containing 0.25 mM ABTS and 0.05 mM H_2O_2 was added to the vial. After some time, the HRP containing layer turned deep bluish-green while the upper cubic phase layer remained colorless. As more time elapsed, the substrate solution on top of the upper cubic phase layer also acquired a bluish-green color. Those results clearly show that the ABTS from the substrate solution diffused through the upper cubic phase layer to the cubic phase layer containing HRP and reacted with the entrapped HRP. The bluish-green colored product then diffused through both of the cubic phase layers into the substrate solution.

Example 5: Enzyme Assays

An assay procedure was developed to test the activity of HRP immobilized in the cubic phase. The immobilized HRP assay involved placing 1 gram of a cubic phase containing HRP at the bottom of a glass vial (12 mm inner diameter). The cubic phase was thoroughly rinsed with buffer solution to remove any surface associated HRP. A 2 mL aliquot of a substrate solution containing 0.25 mM ABTS and 0.05 mM H_2O_2 was poured on top of the cubic phase in the vial and allowed to react for 2 minutes with continuous shaking. At the end of the 2 minutes, the reaction was stopped by decanting the substrate solution into another vial containing 2 mL of a solution containing 2% dodecyl sulfate and 0.1% sodium azide. The concentration of the products was measured spectrophotometrically by determining the absorbance of the solution at 410 nm following appropriate dilution.

A similar assay procedure was used to test the activity of HRP in free solution. The free HRP assay involved preparing a solution of HRP in a 10 mM phosphate

buffer solution (pH 7.4) and a substrate solution of 0.25 mM ABTS and 0.05 mM H_2O_2. Aliquots of each solution were mixed to yield a final volume of 4 mL. The activity of HRP was measured for fixed ABTS and H_2O_2 concentrations with different HRP concentrations, as well as at fixed HRP and H_2O_2 concentrations with different ABTS concentrations. The reaction was allowed to proceed for 2 minutes. At the end of the 2 minutes, 4 mL of a solution containing 2% sodium dodecyl sulfate and 0.1% sodium azide was added to stop the reaction. The concentration of products formed was determined spectrophotometrically by determining the absorbance of the solution at 410 nm following appropriate dilution.

Example 6: Enzyme Stability

The stability of HRP immobilized in the cubic phase was compared to the stability of HRP in free solution. Aliquots of 1 gram pieces of cubic phase containing HRP were submerged in a 10 mM phosphate buffer solution and stored at 37°C. For comparison, a 4 μg/mL solution of HRP in a 10 mM phosphate buffer solution (pH 7.4) was also stored at 37°C. The HRP entrapped in the cubic phase and the HRP in free solution were tested using the assay procedures described above. Each measurement was performed in triplicate. The averages of the three measurements are given in Table 2.

Table 2

Time (days)	Activity of Free HRP	Activity of Immobilized HRP
0	100.0%	100.0%
7	33.0%	51.8%
15	1.7%	23.1%
30	0.0%	4.7%
60	0.0%	1.7%
90	0.0%	0.6%

The results in Table 2 clearly show that the activity of HRP in free solution decreases rapidly with time and is completely lost within 30 days. However, the HRP immobilized in the cubic phase continues to exhibit activity even beyond 90 days. The activity measurements for HRP immobilized in the cubic phase may underestimate the true activity of HRP in the cubic phase since the buffer solution in which the cubic phase was stored was not tested for HRP activity and may contain some HRP which slowly diffused from the cubic phase.

Example 7: Effect of Lipase and Protease Enzymes on Immobilized Enzyme Activity and Matrix Structure

When a GMO-based composition of the invention is injected into a patient's body, the cubic phase formed will be exposed to protease and esterase (lipolytic) enzymes circulating in the body. These enzymes are capable of degrading the entrapped enzyme and the cubic phase, respectively. The effects of trypsin (a protease enzyme) and lipase (an esterase enzyme) on the activity of HRP immobilized in the cubic phase and the cubic phase structure itself were therefore investigated. Twelve aliquots, each containing 1 gram of the cubic phase

containing HRP, were placed in twelve separate glass vials. A first group of three aliquots were then exposed to a 10 mM phosphate buffer solution (pH 7.4) as a control. A second group of three aliquots were exposed to a solution of 0.03 mg/mL trypsin in phosphate buffer. A third group of three aliquots were exposed to a solution of 0.03 mg/mL lipase in phosphate buffer. A fourth group of three aliquots were exposed to a solution of 0.03 mg/mL trypsin and 0.03 mg/mL lipase in phosphate buffer. All of the aliquots were placed in an incubator at 37°C. The solutions in the vials were removed and replaced with fresh solutions every 8 hours. At selected time intervals, the enzyme activity in each of the twelve aliquots of cubic phase was tested using the immobilized HRP assay described above. In addition, the vials were weighed to monitor the degradation of the cubic phase. For comparison, the effect of trypsin on HRP in free solution was also determined. A solution containing 4 μg/mL of HRP and 0.03 mg/mL trypsin in a 10 mM phosphate buffer solution (pH 7.4) was stored in the incubator at 37°C and three aliquots were withdrawn periodically to measure the HRP activity using the assay described above. The results are given in Tables 3 and 4 as the average of three identical measurements.

Table 3

Time (days)	Free HRP	Group 1 (buffer)	Group 2 (trypsin)	Group 3 ((lipase)	Group 4 (t + 1)
	Activity	Activity	Activity	Activity	Activity
0	100.00	100.00	100.00	100.00	100.00
1	33.00	89.77	83.67	94.30	90.13
2	—	83.91	74.60	92.99	81.90
3	—	77.54	68.28	86.87	74.43
5	—	61.80	52.67	71.32	64.50
7	—	51.84	42.45	65.00	55.78

Table 4

Time (days)	Group 1 (buffer)	Group 2 (trypsin)	Group 3 (lipase)	Group 4 (t + l)
	Weight (%)	Weight (%)	Weight (%)	Weight (%)
0	100.00	100.00	100.00	100.00
1	103.27	103.40	110.43	111.63
2	103.67	103.87	108.20	106.57
3	103.57	103.47	100.60	101.43
5	103.30	103.10	94.40	96.70
7	102.80	102.60	88.57	91.60

The activity measurements in Table 3 are plotted in Fig. 2. The data clearly show that only about 33% of the initial HRP activity remains when HRP in free solution is exposed to trypsin for 1 day. In contrast, HRP immobilized in the cubic phase exhibits about 85% activity after 1 day and about 50% activity after 7 days. Further, the observed activity for HRP immobilized in the cubic phase is virtually unaffected by exposure to trypsin, indicating that the cubic phase protects the entrapped enzyme from degradation by protease enzymes in the buffer solution.

The data in Table 4 show that aliquots exposed to the lipase enzyme showed a gradual loss in weight over time, with approximately an 8-12% loss at the end of 7 days. This suggests that the lipase enzymes in the body will slowly biodegrade the cubic phase.

Example 8

Catalase

Catalase catalyzes the breakdown of hydrogen peroxide (H_2O_2) to water and oxygen. H_2O_2 absorbs light at a wavelength of 240 nm and, therefore, catalase activity

can be measured spectrophotometrically by monitoring the decrease in absorption at that wavelength.

To determine if catalase immobilized in the cubic phase of GMO retains its enzymatic activity, catalase in a 50 mM phosphate buffer solution (pH 7.0) was mixed with GMO and allowed to form a cubic phase such that 1 gram of the cubic phase contained 100 units of catalase. Three aliquots, each containing 1 gram of the cubic phase containing catalase, were placed in three separate glass vials along with 1 mL of a 50 mM phosphate buffer solution (pH 7.0) and stored at 37°C. The activity of the immobilized catalase was determined on days 0, 1, 3, 7, 10, and 14 by the following procedure. The cubic phases in the vials were thoroughly rinsed with a 50 mM phosphate buffer solution to remove any surface associated catalase. The cubic phases were then exposed to 3 mL of a 50 mM H_2O_2 and 50 mM phosphate buffer solution (pH 7.0). At 30 and 60 minutes after the addition of the H_2O_2 solution, 0.5 mL aliquots were withdrawn from the vials and replaced with fresh 0.5 mL aliquots of the 50 mM H_2O_2 and 50 mM phosphate buffer solution (pH 7.0). The aliquots were diluted to 2.5 mL with a 50 mM phosphate buffer solution and subjected to spectrophotometric analysis to determine the decrease in the concentration of H_2O_2. In addition, a 300 units/mL solution of catalase in a 50 mM phosphate buffer (pH 7.0) was stored in three separate vials at 37°C. At selected intervals (days 0, 1, 3, 7, 10, and 14), 0.2 mL aliquots were removed and added to 2.3 mL of a solution containing 20 mM H_2O_2 and 50 mM phosphate buffer (pH 7.0). The decrease in the H_2O_2 concentration was monitored over a 1 minute period using a spectrophotometer set at a wavelength of 240 nm. The results are given in Table 5 and shown in Fig. 3.

Table 5

Time (days)	Activity of Free Catalase (%)	Activity of Immobilized Catalase (%)
0	100.00	100.00
1	90.07	92.08
3	62.48	78.83
7	33.06	73.02
10	8.23	57.34
14	1.32	49.15

The results in Table 5 and Fig. 3 clearly show that catalase is successfully immobilized in the cubic phase with retention of activity. A 50% decrease in the activity of the immobilized catalase is observed over a 14 day period. In comparison, the activity of the free catalase in solution decreased by about 99% in 14 days. In addition, the absence of catalase in the buffer solution suggests that catalase (250,000 Da) does not diffuse through the aqueous channels in the cubic phase.

Example 9

Human Cytochrome P450

The cytochrome P450 family consists of membrane bound enzymes that metabolize many chemicals in the body. CYP-2A6 is a coumarin 7-hydroxylase enzyme: it converts coumarin (substrate) to 7-hydroxycoumarin (product). The rate of formation of the product can be measured fluorometrically with excitation at 390 nm and emission at 440 nm. In this example, it was determined whether a complex enzyme system comprising CYP-2A6, CYP-r and glucose-6-phosphate dehydrogenase (G6PDase) could be immobilized in a GMO cubic phase with retention of enzymatic activity.

A microsomal preparation containing CYP-2A6 and CYP-r from Gentest Corp. (catalog #M104r, 10 mg/mL protein) was stored at -80°C and used as received after rapid thawing in a 37°C water bath. An aliquot of enzyme solution (0.2 mL) was stored at 37°C and 20 μL aliquots were used to measure enzyme activity at selected intervals by a method provided by Gentest Corp. In the method, a 1 mL reaction mixture containing 0.2 mg/mL CYP-2A6/CYP-r protein (20 μL aliquot of the solution received), 1.3 mM NADP+, 3.3 mM glucose-6-phosphate (G6P), 0.4 U/mL G6PDase, 3.3 mM $MgCl_2$ and 0.4 mM coumarin in 50 mM Tris (pH 7.4) was incubated at 37°C for 15 min. After incubation, the reaction was stopped by the addition of 0.2 mM 20% trichloroacetic acid and centrifuged (10,000 x g) for 1 minute. Two hundred microliters of the supernatant was added to 3.8 mL of 100 mM Tris (pH 9.0) and the fluorescence was determined as described above. Activity was quantitated against a standard curve for 7-hydroxycoumarin.

Enzyme solutions in 50 mM Tris (pH7.4) were added to GMO to form the cubic phase such that 1 gram of matrix contained 2 mg CYP-2A6/CYP-r protein and 20 units of G6PDase. One gram matrix (in duplicate) was placed in a glass vial along with 1 ml 50 mM Tris (pH 7.4) solution. At selected intervals, the matrix was rinsed thoroughly with fresh Tris solution to remove surface associated enzymes, if present. The matrix was then incubated at 37°C for 15 min with 1 ml substrate solution containing 1.3 mM NADP+, 3.3 mM G6P, 0.4 U/mL G6PDase, 3.3 mM $MgCl_2$ and 0.4 mM coumarin in 50 mM Tris (pH 7.4). After incubation, the reaction solution was decanted into another vial containing 0.2 mM trichloroacetic acid to terminate the reaction. The solution was centrifuged, diluted and analyzed for product as described above. Results are shown in Table 6.

Table 6
Cytochrome P-450 2A6 Activity (n = 2)

Time	Activity of free CYP-2A6 in solution at 37°C			
(hours)	Average Activity	Std. dev.	% Activity	Std. dev.
	(pmole/min x mg protein)			
0	259.98	8.28	100.00	3.18
3	182.47	16.88	70.19	6.49
6	95.33	3.39	36.67	1.31
18	57.76	3.87	22.22	1.49
48	14.96	1.16	5.75	0.45
84	1.64	0.36	0.63	0.14

Time	Activity of immobilized CYP-2A6 at 37°C			
(hours)	Average Activity	Std. dev.	% Activity	Std. dev.
	(pmole / min x g gel)			
0	1.43	0.36	100.00	25.11
3	2.02	0.54	141.79	37.84
6	1.40	0.05	97.76	3.72
18	1.18	0.02	82.80	1.09
48	1.43	0.24	100.42	16.99
84	1.13	0.35	79.05	24.32

As can be seen in Table 6, the activity of the enzyme system in free buffer solution decreased rapidly, with near complete loss of activity in 3.5 days. In contrast, the enzyme system entrapped within the GMO cubic phase continued to exhibit enzymatic activity beyond 3.5 days, indicating successful immobilization and enhanced stability in the cubic phase matrix. Moreover, it is likely that the protocol described above actually underestimated the true stabilizing influence of the cubic phase matrix on the enzymes entrapped within. Experiments have shown that the substrate and product molecules partition into the cubic phase from the aqueous phase. Since the product concentrations were measured by sampling the surrounding aqueous medium, any product that had partitioned with the cubic phase escaped measurement. Thus, the activity of the immobilized cytochrome P450 enzyme system is probably significantly higher than that observed.

In conclusion, the Examples indicate that enzymes, such as HRP, catalase, and a mixture of CYP-2A6, CYP-r, and G6PDH, entrapped in a cubic phase of GMO are effectively retained within the cubic phase and remain stable and active over a considerably longer period of time than the same enzyme in free solution.

It will be recognized by those skilled in the art that changes or modifications may be made to the above-described embodiments without departing from the broad inventive concepts of the invention. It should therefore be understood that this invention is not limited to the particular embodiments described herein, but is intended to include all changes and modifications that are within the scope and spirit of the invention as set forth in the claims.

WHAT IS CLAIMED IS:

1. A composition for administration of a bio-affecting catalyst, said composition comprising at least one said bio-affecting catalyst retained within a matrix, said matrix substantially preventing release of said catalyst for a pre-determined time period, said matrix enabling fluid communication between said catalyst and an external medium in which said composition is disposed, said fluid communication resulting in accessibility of said catalyst to substrates disposed within said external medium, and release into said external medium of products formed by said catalyst.

2. The composition of claim 1, wherein said bio-affecting catalyst is selected from the group consisting of enzymes, enzyme derivatives, peptides and peptide derivatives.

3. The composition of claim 1, wherein said bio-affecting catalyst is combined with an anchor molecule.

4. The composition of claim 1, which erodes substantially after expiration of said pre-determined time period.

5. The composition of claim 1, which is formable as a low viscosity phase mixture comprising said bio-affecting catalyst and at least one matrix-forming compound, and is inducible to undergo a phase transition to a high viscosity phase to produce said matrix with said bio-affecting catalyst retained therein.

6. The composition of claim 5, wherein said phase transition is inducible by exposing said low

viscosity phase mixture to conditions selected from the group consisting of:

a) temperature change;

b) pH change;

c) solvent change; and

d) a combination of any or all of a), b) and c).

7. The composition of claim 6, wherein said phase transition is inducible in response to increasing temperature from between about 18-25°C to about 30-40°C.

8. The composition of claim 6, wherein said phase transition is inducible upon introduction of said low viscosity phase mixture into a living mammalian body.

9. The composition of claim 5, wherein said high viscosity phase is a gel.

10. The composition of claim 5, wherein said high viscosity phase is a liquid crystalline phase, which comprises channels through which said fluid communication occurs.

11. The composition of claim 10, wherein said liquid crystalline phase is a cubic phase.

12. The composition of claim 10, wherein said channels have an average cross-sectional diameter of between about 0.1 nm and about 100 nm.

13. The composition of claim 12, wherein said channels have an average cross-sectional diameter of between about 1 nm and about 20 nm.

14. The composition of claim 10, wherein said low viscosity phase mixture comprises at least one

amphiphilic matrix-forming compound and said at least one bio-affecting catalyst.

15. The composition of claim 14, wherein said amphiphilic matrix-forming compound is selected from the group consisting of lipids, surfactants and soaps.

16. The composition of claim 15, wherein said lipid is a glyceride.

17. The composition of claim 16, wherein said glyceride is glyceryl monooleate.

18. The composition of claim 17, wherein said low viscosity phase mixture comprises by weight between about 60% and about 99.99% glyceryl monooleate, up to about 40% of an aqueous phase, and between about 0.01% and 40% of said bio-affecting catalyst.

19. The composition of claim 18, wherein said low viscosity phase mixture comprises by weight about 85% of glyceryl monooleate, about 14.87% of said aqueous phase, and about 0.13% of said bio-affecting catalyst.

20. A composition for administration of a bio-affecting catalyst, said composition comprising at least one said bio-affecting catalyst retained within a high viscosity cubic phase matrix that substantially prevents release of said catalyst for a pre-determined time period, said cubic phase having fluid communication channels enabling accessibility of said catalyst to substrates disposed within an external fluid medium in which said composition is disposed, and release into said external medium of products formed by said catalyst, said composition being formable as a low viscosity phase mixture comprising said bio-affecting catalyst, and at least one cubic phase-forming compound, said mixture

being inducible to undergo a phase transition to said cubic phase matrix with said bio-affecting catalyst retained therein.

21. The composition of claim 20, wherein said bio-affecting catalyst is selected from the group consisting of enzymes, enzyme derivatives, peptides and peptide derivatives.

22. The composition of claim 20, wherein said bio-affecting catalyst is combined with an anchor molecule.

23. The composition of claim 20, which is erodible substantially after expiration of said pre-determined time period.

24. The composition of claim 20, wherein said channels have an average cross-sectional diameter of between about 0.1 nm and about 100 nm.

25. The composition of claim 24, wherein said channels have an average cross-sectional diameter of between about 1 nm and about 20 nm.

26. The composition of claim 20, wherein said phase transition is inducible by exposing said low viscosity phase mixture to conditions selected from the group consisting of:

a) temperature change;
b) pH change;
c) solvent change; and
d) a combination of any or all of a), b) and c).

27. The composition of claim 26, wherein said phase transition is inducible in response to increasing temperature from between about 18-25°C and about 30-40°C.

28. The composition of claim 26, wherein said phase transition is inducible upon introduction of said low viscosity phase mixture into a living mammalian body.

29. The composition of claim 20, wherein said cubic phase-forming compound is selected from the group consisting of lipids, surfactants and soaps.

30. The composition of claim 29, wherein said lipid is a glyceride.

31. The composition of claim 30, wherein said glyceride is glyceryl monooleate.

32. The composition of claim 31, wherein said low viscosity phase mixture comprises by weight between about 60% and about 99.99% glyceryl monooleate, up to about 40% of an aqueous phase, and between about 0.01% and 40% of said bio-affecting catalyst.

33. The composition of claim 32, wherein said low viscosity phase mixture comprises by weight about 85% of glyceryl monooleate, about 14.87% of said aqueous phase, and about 0.13% of said bio-affecting catalyst.

34. A composition comprising at least one bio-affecting catalyst retained within a high viscosity cubic phase matrix that substantially prevents release therefrom of said catalyst for a pre-determined time period, said composition comprising by weight between about 60% and about 99.99% of glyceryl monooleate, up to about 40% of an aqueous phase, and between about 0.01%

and about 40% of said at least one bio-affecting catalyst.

35. A method for supplying a bio-affecting catalyst to a patient, comprising the steps of:

a) providing a composition comprising at least one said bio-affecting catalyst retained within a matrix, said matrix substantially preventing release of said catalyst for a pre-determined time period, said matrix enabling fluid communication between said catalyst and an external medium in which said composition is disposed, said fluid communication resulting in accessibility of said catalyst to substrates disposed within said external medium, and release into said external medium of products formed by said catalyst; and

b) administering said composition to said patient, thereby supplying said bio-affecting catalyst to said patient.

36. The method of claim 35, wherein said composition is provided by:

i) preparing a low viscosity phase mixture comprising said bio-affecting catalyst and at least one matrix-forming compound; and

ii) inducing said mixture to undergo a phase transition to a high viscosity phase to produce said matrix with said bio-affecting catalyst retained therein.

37. The method of claim 36, wherein said phase transition is induced upon introduction of said mixture into said patient's body, and said composition is administered by injection into said patient's body.

38. The method of claim 36, wherein said phase transition is induced prior to administration to said patient.

39. The method of claim 38, wherein said composition is administered by implantation into said patient's body.

40. The method of claim 38, wherein said composition is administered by removing a component from said patient's body, exposing said component to said composition, and returning said component to said patient's body.

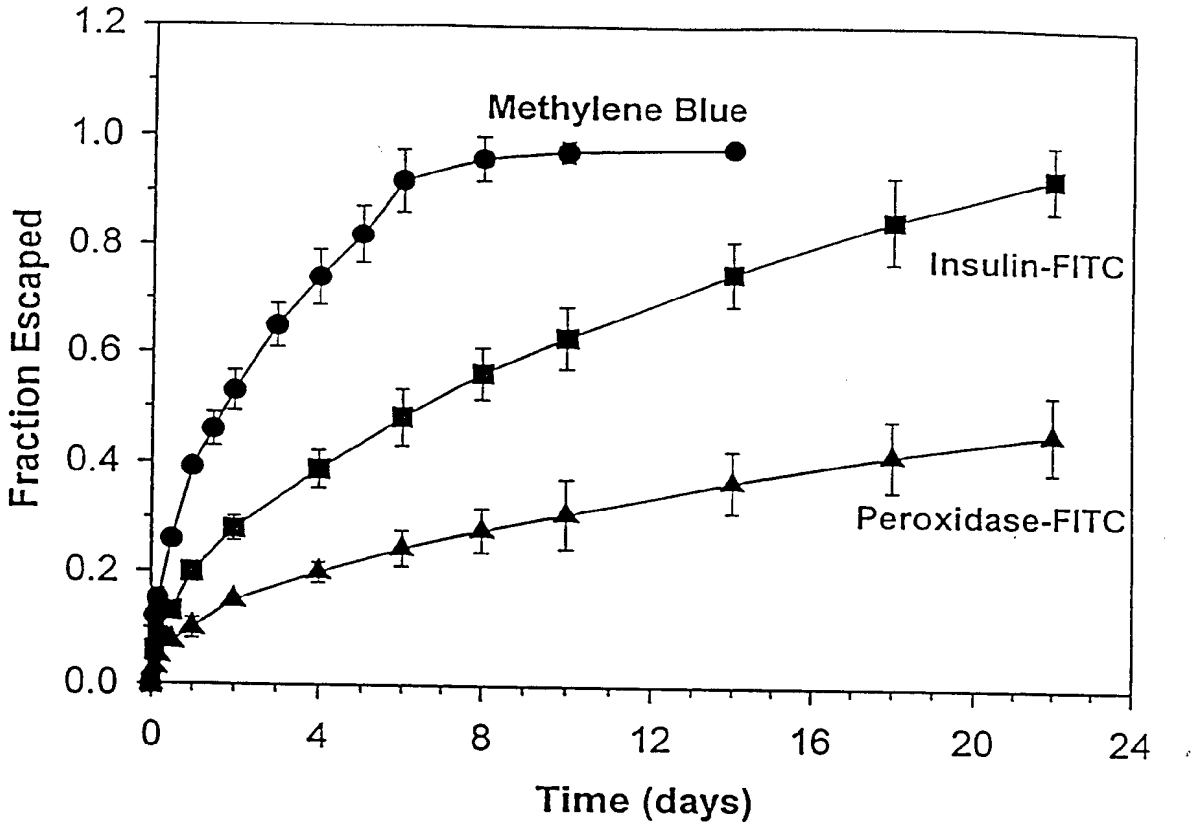
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Fraction Escaped
Methylene Blue
Insulin-FITC
Peroxidase-FITC
0
4
8
12
16
20
24
Time (days)

Figure 1

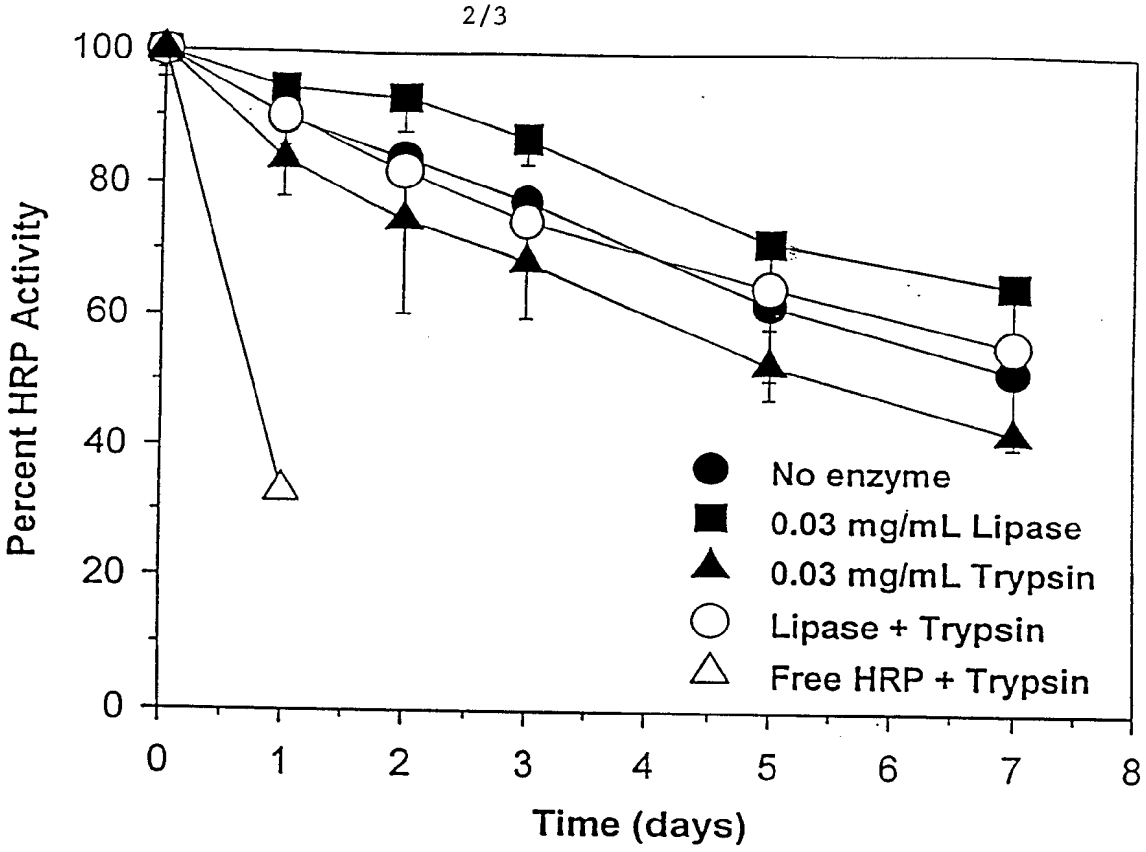
Percent HRP Activity
100
80
60
40
20
0
0
1
2
3
4
5
6
7
8
Time (days)
No enzyme
0.03 mg/mL Lipase
0.03 mg/mL Trypsin
Lipase + Trypsin
Free HRP + Trypsin

Figure 2

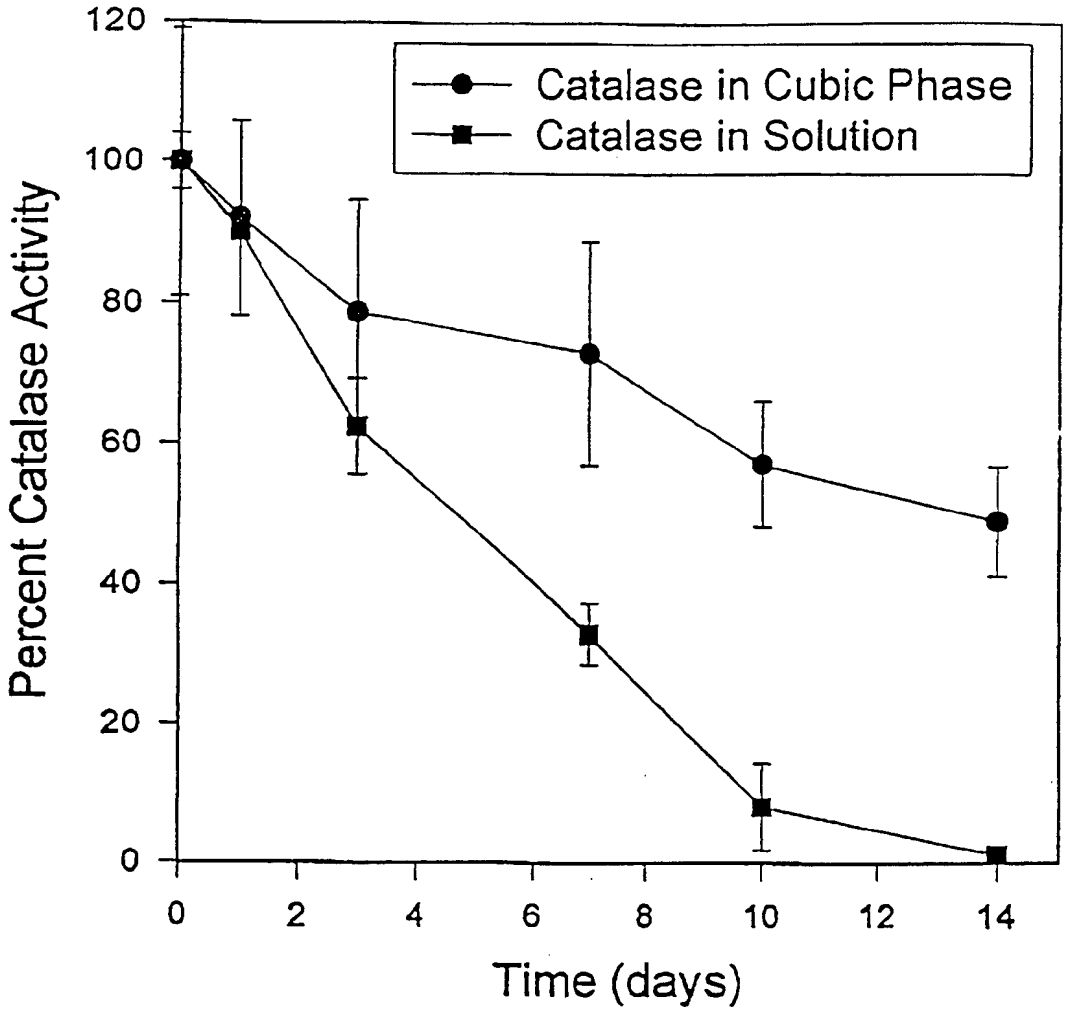
Catalase in Cubic Phase
Catalase in Solution
Percent Catalase Activity
Time (days)
120
100
80
60
40
20
0
0
2
4
6
8
10
12
14

Figure 3

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/09262

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : A61K 9/14, 47/30
US CL : 424/486; 514/772.3, 785, 944
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/486; 514/772.3, 785, 944

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, 5,162,430 A (RHEE ET AL.) 10 November 1992, see column 3, lines 17-52, and claims.	1-40

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 09 AUGUST 1996	Date of mailing of the international search report 05 SEP 1996
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